

APPENDIX A

**"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE
CLAIMS OF 09/758,962 WITH ENTRY OF THIS AMENDMENT**

1. A recombinant plant viral vector comprising a polynucleotide, which polynucleotide comprises [comprising]: [(1)] an IRES nucleotide sequence, [(2)] an ORF encoding a peptide of interest, and [(3)] an ORF encoding a viral protein, where the IRES nucleotide sequence[(1)] is located between the peptide of interest ORF [(2)] and the viral protein ORF[(3)].

2. The vector[polynucleotide] according to claim 1 wherein a promoter 5' to the IRES sequence, the peptide of interest ORF, and the viral protein ORF[(1), (2) and (3)] promotes transcription of[transcribes] a mRNA containing said polynucleotide [(1), (2) and (3)].

3. The vector[polynucleotide] according to claim 2 wherein the IRES nucleotide sequence is a naturally occurring IRES or a fragment of a naturally occurring IRES that can direct translation of the peptide of interest ORF [(2)] or the viral protein ORF [(3)].

4. The vector[polynucleotide] according to claim 2 wherein the IRES sequence comprises a nucleotide sequence of: SEQ ID NO: 1, [, SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7;]or a fragment of SEQ ID NO: 1[, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6, or SEQ ID NO: 7], that can direct translation of the peptide of interest ORF or the viral protein ORF [(2) or (3)].

5. The vector[polynucleotide] according to claim 2 wherein the viral protein is a coat protein.

6. Cancelled.

7. A recombinant virus comprising a recombinant viral vector according to claim 5.

8. A host comprising a recombinant virus according to claim 7[6].
9. An IRES capable of directing the expression of an internal ORF in a heterologous viral vector.
10. An IRES according to claim 9 wherein the IRES is a IRES_{cp}.
11. An IRES according to claim 10 wherein the IRES is crTMV IRES_{cp}.
12. A viral vector construct that expresses a bicistronic mRNA comprising an ORF positioned upstream of an IRES sequence and followed by a coat protein coding sequence.
13. A viral vector construct according to claim 12 wherein the ORF encodes a native or foreign gene.
14. A viral vector construct according to claim 53[13] wherein the reporter gene encodes a green fluorescent protein.
15. A viral vector construct, comprising: [(1)] a viral genome, and [(2)] an IRES sequence, wherein the IRES sequence is heterologous to the viral genome, wherein the IRES sequence is downstream of a desired gene or ORF and upstream of a virus coat protein gene, wherein the IRES sequence is in the sense or antisense orientation.
16. A viral vector construct according to claim 15 wherein the viral vector construct expresses a bicistronic mRNA.
17. A viral vector construct according to claim 15 wherein the viral genome is the genome of potato virus X.

18. A potato virus X-based viral vector construct comprising the viral vector construct according to claim 15, wherein the potato virus X-based viral vector construct gives rise to single cell infection sites.

19. A viral vector construct according to claim 15 further comprising [(3)] a stable stem loop structure inserted 5' of the IRES sequence.

20. A viral vector construct according to claim 19 wherein the stem loop structure is immediately upstream of the IRES sequence.

21. A viral vector construct according to claim 20 wherein the stem loop structure causes a reduction in the expression of the virus coat protein gene.

22. A viral vector construct according to claim 21 wherein the stem loop structure interferes with direct interaction of a ribosome at the IRES sequence.

23. A viral vector construct according to claim 15 further comprising [(3)] a stable stem loop structure inserted 3' of the IRES sequence.

24. A viral vector construct according to claim 23 wherein the stem loop structure prevents expression of the virus coat protein gene.

25. A viral vector construct according to claim 23 wherein the stem loop structure effectively blocks scanning ribosomes.

26. A viral vector comprising a **natural or modified plant virus**[plant virus-derived] IRES sequence linked to **an**[the] ORF encoding a protein of interest, wherein **said**[the plant virus-derived] IRES sequence directs translation of the ORF and wherein the protein of interest is heterologous to the viral vector.

27. A viral vector according to claim 26 wherein said[the plant virus-derived]IRES sequence initiates translation effectively in either sense or antisense orientation.

28. A viral vector according to claim 27 wherein said[the plant virus-derived]IRES sequence is an IRES_{Scp} sequence.

29. A viral vector construct comprising the function of producing a bicistronic subgenomic RNA in which two ORFs are separated by an IRES.

30. A recombinant plant viral vector construct comprising a modified IRES sequence that directs higher levels of protein expression.

31. Cancelled.

32. Cancelled.

33. Cancelled.

34. Cancelled.

35. Cancelled.

36. Cancelled.

37. Cancelled.

38. A polynucleotide comprising pIRESs-XCP.

39. Cancelled.

40. Cancelled.

41. Cancelled.

42. Cancelled.

43. Cancelled.

44. Cancelled.

45. Cancelled.

46. Cancelled.

47. Cancelled.

48. Cancelled.

49. Cancelled.

50. Cancelled.

51. Cancelled.

52. Cancelled.

53. A viral vector construct according to Claim 12, further comprising a reporter gene.

54. A recombinant or isolated polynucleotide comprising: an IRES nucleotide sequence, an ORF encoding a peptide of interest, and an ORF encoding a viral protein, wherein the IRES nucleotide sequence is located between the peptide of interest ORF and the

viral protein ORF and wherein the IRES nucleotide sequence is heterologous to the viral protein ORF.

55. The polynucleotide of claim 54, wherein one or more of the IRES nucleotide sequence or the viral protein ORF comprises a tobamovirus nucleotide sequence.

56. The polynucleotide of claim 55, wherein the tobamovirus comprises crTMV.